KPL Streptavidin, HRP-Labeled

molecular biology grade

Catalog No.	Size
5950-0004 (474-3000)	1 mL

DESCRIPTION

Streptavidin is a 60,000 dalton protein isolated from the bacterium *Streptomyces avidinii*. Streptavidin has been shown to bind four molecules of biotin with high affinity ($K_D=10^{-14}$ M) [1]. Electrophoretically pure streptavidin is linked to horseradish peroxidase using a modification of the periodate method of Nakane and Kawaoi [2].

FORM

The conjugate is provided in a liquid buffer containing a proprietary stabilizer and an anti-bacterial agent. It is prepared with molecular biology grade chemicals and dispensed into RNase/DNase-free sterile vials.

STORAGE/STABILITY

Store at 2-8°C. Stable for a minimum of 1 year from date of receipt at 2-8°C as an undiluted liquid. Dilute immediately before use.

ENZYME:PROTEIN RATIO

Molar peroxidase: streptavidin ratio = 2.5:1

CONCENTRATION

The concentration of streptavidin is 0.1 mg/mL in a 1.0 mL volume.

APPLICATIONS

KPL Peroxidase-Labeled Streptavidin is suitable for use in Northern blotting, Southern blotting, plaque and colony hybridizations, in situ hybridization, and immunohistochemistry (brief protocols described below) applications. This conjugate may also be used for ELISA and immunoblotting procedures (See References 3-6).

SUGGESTED WORKING DILUTIONS

Different assay conditions require that serial dilutions of all reagents be performed to determine optimal working concentrations. Prepare the working dilution immediately before use. Long-term storage at a working dilution may result in enzyme inactivation and performance loss. Do not use sodium azide in the diluent. For suggested starting dilutions, see the appropriate protocol.

SUGGESTED PROTOCOLS

All steps are at room temperature unless otherwise noted.

Southern Blotting, Northern Blotting, Plaque and Colony Hybridizations

Following hybridization with a biotinylated probe and post-hybridization washing:

1. Place membrane in a small container or hybridization bag and block with KPL Detector Block, or other appropriate blocking solution, for 30 minutes.
2. Dilute KPL peroxidase-labeled streptavidin 1:100-1:1000 in blocking solution, using at least 0.25 mL per cm^2 membrane. Incubate membrane for 20 minutes.
3. Transfer membrane to a clean container and wash with KPL Biotin Wash Solution). Wash 3 times for 5 minutes each using at least 0.4 mL wash solution per cm^2 membrane.
4. Detect using KPL TMB Membrane Substrate, KPL LumiGLO® Chemiluminescent Substrate or other appropriate peroxidase substrate following manufacturer's protocols.

In Situ Hybridization

1. Following hybridization of tissue or cells with a biotinylated probe and post-hybridization washing:
2. Dilute KPL peroxidase-labeled streptavidin 1:20-1:200 in an appropriate diluent and apply approximately 100 µL to specimen. Cover slide to prevent evaporation. Incubate in a 37°C humidified chamber for 20 minutes. The optimal dilution of KPL HRP-SA must be determined experimentally.
3. Immerse slide in a Coplin jar containing KPL Biotin Wash Solution, or other appropriate wash solution. Wash 3 times for 5 minutes each.
4. Detect using KPL TrueBlue™ or other appropriate peroxidase substrate following manufacturer's instructions.
5. Apply a red counterstain, such as Orcein according to manufacturer's instructions.
6. Dehydrate and mount the slide with an organic mounting media such as Permount.

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Immunohistochemistry

Following incubation of the specimen with primary and biotin-labeled secondary antibody:

1. Dilute KPL Peroxidase-Labeled Streptavidin 1:10-1:100 in KPL 10% Normal Goat Serum. Flood the slide with diluted KPL Peroxidase-Labeled Streptavidin. Incubate for 30 minutes.
2. Soak the slide in PBS or Tris-HCl for 5 minutes.
3. Detect using KPL TrueBlue or other appropriate peroxidase substrate, and counterstain if desired.

PRODUCT SAFETY AND HANDLING

This product is considered non-hazardous as defined by the Hazard Communication Standard (29 CFR 1910.1200). Avoid contact with skin and eyes. In case of contact or spillage, clean with copious amounts of water. Product may be disposed via a sanitary sewer.

REFERENCES

1. Holmberg, A., et al. (2005). Electrophoresis, Feb;26(3):501-10.
2. Nakane, P.K. and Kawaoi, A. (1974). J. Histochem. Cytochem. 22: 1084-1091.
3. Brigati, D.J., et al. (1983) Virology, 126: 32-50.
4. Harlow, E. and Lane, D. (1988) Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory Press, NY.
5. Sambrook, J., et al. (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, NY.
6. Crowther, J.R. (1995) Methods in Molecular Biology: ELISA: Theory and Practice. Vol. 42, Humana Press, NJ.

RELATED PRODUCTS	CAT. NO.
KPL 5X Detector Block	5920-0004 (71-83-00)
KPL 10X Biotin Wash Solution	5960-0015 (50-63-06)
KPL 10% Normal Goat Serum	5560-0007 (71-00-27)
KPL LumiGLO Chemiluminescent Substrate	5430-0042 (54-61-02)
KPL TMB 1-Component Membrane Substrate	5420-0029 (50-77-18)
KPL TrueBlue	5510-0030 (50-78-02)

The product listed herein is for research use only and is not intended for use in human or clinical diagnosis.

For assistance, contact LGC SeraCare at 508.244.6400.

The package insert for this panel can be found at www.seracare.com.

A printed copy of the package insert or data sheet may be requested by email at CDx-Info@LGCGroup.com or by phone at 508.244.6400.